

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of the claims in the application.

Listing of Claims:

1. (previously presented) A method for purifying biomolecules, and/or protein complexes, the method comprising:

(a) providing an expression environment containing one or more heterologous nucleic acids encoding one or more subunits of a biomolecule complex;

(b) maintaining the expression environment under conditions that facilitate expression of the one or more subunits in a native form as fusion proteins with subunits being fused to at least two different affinity tags, wherein one of the affinity tags consists of one or more IgG binding domains of Staphylococcus protein A; and

(c) purifying the one or more subunits by a combination of at least two different affinity purification steps each comprising binding the one or more subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the one or more subunits from the support material after substances not bound to the support material have been removed to provide a purified biomolecule and/or protein complex.

2. (previously presented) A method for purifying biomolecule and/or protein complexes, the method comprising:

(a) providing an expression environment containing one or more heterologous nucleic acids encoding at least two subunits of a biomolecule complex;

(b) maintaining the expression environment under conditions that facilitate expression of the two or more subunits in a native form as fusion proteins with subunits being fused to at least two different affinity tags, wherein one of the affinity tags consists of one or more IgG binding domains of Staphylococcus protein A; and

(c) purifying the complex by a combination of at least two different affinity purification steps each comprising binding the two or more subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the complex from the support material after substances not bound to the support material have been removed to provide a purified biomolecule and/or protein complex.

7/2/05

no support limit

30 04/10/04/3

11/23/03

5/5/05

8/11/01/653

wo/a/96/40943

3. (previously presented) The method according to claim 1 or 2, wherein between the one or more subunits and one or more of the affinity tags a specific proteolytic cleavage site is present in one or more of the fusion proteins which facilitates the removal of one or more of the affinity tags.

4. (previously presented) The method according to claim 3, wherein the specific proteolytic cleavage site is an enzymatic cleavage site.

5. (previously presented) The method according to claim 4, wherein the specific proteolytic cleavage site is the cleavage site for Tobacco Etch Virus protease NIA.

6. (previously presented) The method according to claim 5, wherein the one or more of the proteolytic cleavage site is used to cleave the one or more of the subunits in step (c) from the IgG binding domain of Staphylococcus protein A bound to the support material.

7. (previously presented) The method according to claim 6, wherein the affinity purification of step (c) comprises:

(i) binding the one or more subunits via the one or more IgG binding domains of Staphylococcus to a support material capable of specifically binding the latter, removing substances not bound to the support material and separating the one or more subunits from the support material by cleaving off the IgG binding domains via the specific proteolytic cleavage site, and

(ii) binding the subunit via another affinity tag to a second support material capable of specifically binding the latter, removing substances not bound to the support material and separating the polypeptide or subunit from the support material.

8. (previously presented) The method according to claim 7, wherein step (ii) is carried out before step (i).

9. (previously presented) The method according to claim 8, wherein the fusion protein contains a second specific proteolytic cleavage site for the removal of one or more of the other affinity tags.

10. (previously presented) The method according to claim 9, wherein one of the affinity tags consists of at least one calmodulin binding peptide.

11. (previously presented) The method according to claim 10, wherein a chemical agent is used to separate the one or more polypeptides or subunits from the support material.

12. (previously presented) The method according to claim 1 or 2, wherein the purified biomolecule and/or protein complex is detected.